

CLAIMS

1. A polynucleotide comprising:

(a) a region which comprises as operably linked components (i) a promoter which provides for seed preferred expression; and (ii) a nucleotide sequence derived from a bacterium which sequence encodes a carotene desaturase; and (iii) a transcription termination region; and

(b) a further region which comprises as operably linked components (i) a promoter which provides for seed preferred expression; and (ii) a nucleotide sequence encoding a phytoene synthase which sequence is derived from maize (*Zea sp.*) or rice (*Orzya sp.*); and (iii) a transcription termination region.

2. A polynucleotide according to claim 1 wherein the sequence which encodes the carotene desaturase is derived from *Erwinia sp.*

3. A polynucleotide according to claim 1 or claim 2 wherein said promoter is selected from the Glutelin 1 promoter and the Prolamin promoter and said transcription termination region is selected from the Nos; CaMV 35S and PotP1-II transcription termination regions.

4. A polynucleotide according to any one of claims 1 to 3 wherein the sequence which encodes carotene desaturase and the sequence which encodes phytoene synthase further comprises a sequence encoding a plastid targeting sequence.

5. A polynucleotide according to any one of claims 1 to 4 wherein said region and/or said further region further comprises an intron.

6. A polynucleotide according to any one of claims 1 to 5 which comprises a sequence selected from the group depicted as SEQ ID NO: 1; 2; 3; 4; and 6.

7. A polynucleotide sequence which is the complement of one which hybridises to a polynucleotide according to claim 6 at a temperature of about 65°C in a solution containing 6 x SSC, 0.01% SDS and 0.25% skimmed milk powder, followed by rinsing at the same temperature in a solution containing 0.2 x SSC and 0.1% SDS

- 46 -

wherein said polynucleotide sequence still comprises a region encoding a carotene desaturase and a further region encoding a phytoene synthase and when said polynucleotide sequence is inserted into plant material the seed of a plant regenerated from said material produce an increased amount of carotenoids when compared to a control like-seed.

8. A polynucleotide sequence according to claim 7 wherein when said polynucleotide sequence is inserted into plant material, the seed of a plant regenerated from said material produces at least a sixty fold increase in carotenoids when compared to a control like-seed.
9. A polynucleotide sequence according to claim 7 wherein when said polynucleotide sequence is inserted into plant material, the seed of a plant regenerated from said material produces at least a three hundred and fifty fold increase in carotenoids when compared to a control like-seed.
10. A polynucleotide sequence according to claim 7 wherein when said polynucleotide sequence is inserted into plant material the seed of a plant regenerated from said material produces carotenoids at a level of at least 10µg/g of endosperm of said seed.
11. A polynucleotide sequence according to claim 7 wherein when said polynucleotide sequence is inserted into plant material the seed of a plant regenerated from said material produces carotenoids at a level of at least 15µg/g of endosperm of said seed.
12. A polynucleotide sequence which is the complement of one which hybridises to a polynucleotide according to claim 6 at a temperature of about 65°C in a solution containing 6 x SSC, 0.01% SDS and 0.25% skimmed milk powder, followed by rinsing at the same temperature in a solution containing 0.2 x SSC and 0.1% SDS wherein said polynucleotide sequence still comprises a region encoding a carotene desaturase and a further region encoding a phytoene synthase and when said polynucleotide sequence is inserted into plant material the seed of a plant

regenerated from said material produce carotenoids amounting to at least 80% of the carotenoid content of a seed which comprises a polynucleotide selected from the group depicted as SEQ ID NO: 1; 2; 3; 4; 5 and 6.

- 5 13. A polynucleotide sequence according to claim 12 wherein when said polynucleotide sequence is inserted into plant material the seed of a plant regenerated from said material produces carotenoids amounting to at least 100% of the carotenoid content of a seed which comprises a polynucleotide selected from the group depicted as SEQ ID NO: 1; 2; 3; 4; 5 and 6.
- 10 14. A polynucleotide sequence according to any one of claims 7 to 13 wherein said seed is a rice seed.
- 15 15. A polynucleotide or a polynucleotide sequence according to any one of claims 1 to 14 which further comprises a region which encodes a selectable marker.
16. A polynucleotide or a polynucleotide sequence according to claim 15 wherein said selectable marker comprises a mannose-6-phosphate isomerase gene.
- 20 17. A polynucleotide or a polynucleotide sequence according to any one of claims 1 to 16 which is codon optimised for expression in a particular plant species.
18. A polynucleotide or a polynucleotide sequence according to claim 17 wherein said plant species is rice (*Orzya sp.*).
- 25 19. A vector comprising a polynucleotide or a polynucleotide sequence according to any one of claims 1 to 18.
- 30 20. A method for increasing the carotenoid content of seeds comprising inserting into plant material a polynucleotide or a polynucleotide sequence according to any one of claims 1 to 18 or a vector according to claim 19; and regenerating a seed-containing plant from said material and identifying the seeds which contain carotenoids at levels greater than those of control like-seeds.

21. A method for increasing the carotenoid content of a seed comprising inserting into plant material a polynucleotide comprising a sequence selected from the group depicted as SEQ ID NO: 1; 2; 3; 4; 5 and 6 and regenerating a seed-containing plant from said material and identifying the seed which contains carotenoids at levels greater than those of a control like-seed.
22. A method according to claim 20 or claim 21 wherein said seed contains at least a sixty fold increase in carotenoids when compared to a control like-seed.
23. A method according to claim 22 wherein said seed contains at least a three hundred and fifty fold increase in carotenoids when compared to control like-seed.
24. A method according to claim 20 or claim 21 wherein said seed contains carotenoids at a level of at least 10 μ g/g of endosperm of said seed.
25. A method according to claim 24 wherein said seed contains carotenoids at a level of at least 15 μ g/g of endosperm of said seed.
26. A method according to any one of claims 20 to 25 wherein said carotenoids are selected from the group consisting of: lycopene; alpha-carotene; lutein; beta-carotene; zeaxanthin; antheraxanthin; violaxanthin; and neoxanthin or a combination thereof.
27. A seed obtained by a method according to any one of claims 20 to 26.
28. A seed according to claim 27 which is a rice seed.
29. A plant which comprises a seed according to claim 27 or claim 28.

30. A plant or plant material which comprises a polynucleotide or a polynucleotide sequence according to any one of claims 1 to 18 or a vector according to claim 19.

5 31. A plant or plant material according to claim 30 which is a rice plant or is rice plant material.

32. A plant or plant material according to claim 30 which is a maize plant or is maize plant material.

10 33. A plant according to any one of claims 29 to 32 which further comprises a polynucleotide which provides for a trait selected from the group consisting of: insect resistance and/or tolerance; nematode resistance and/or tolerance; herbicide resistance and/or tolerance; improved resistance and/or tolerance to stress; a
15 substance having pharmaceutical activity; and any other desired agronomic trait.

34. Use of a polynucleotide or a polynucleotide sequence according to any one of claims 1 to 18 or a vector according to claim 19 in a method for the production of seeds containing increased carotenoids.

20 35. Use of a polynucleotide selected from the group depicted as SEQ ID NO: 1; 2; 3; 4; 5 and 6 for the production of a seed which contains carotenoids at levels greater than those of a control like-seed.

25 36. Use of a polynucleotide or a polynucleotide sequence according to any one of claims 1 to 18 or a vector according to claim 19 in a method for the production of a plant comprising said polynucleotide, said polynucleotide sequence or said vector.

30 37. Use of a polynucleotide selected from the group depicted as SEQ ID NO: 1; 2; 3; 4; 5 and 6 in a method for the production of a plant comprising said polynucleotide.